

In the Specification (clean copy as amended)

Page 6, third full paragraph:

A1 The cells expressing the potassium transport protein may transiently express the protein or constitutively express the protein. The cells may be of any type which can express the protein in appropriate conformation to allow for the transport of potassium. Examples of such cells include, mammalian cells, vertebrate cells, and invertebrate cells. Examples of mammalian cells suitable for use in the invention include, but are not limited to, cells of neuronal origin, fibroblasts, myocardial cells, COS cells, Chinese hamster ovary (CHO) cells, embryonic kidney cells, fibroblasts, HeLa cells, and the like. Examples of suitable non-mammalian vertebrate cells include, but are not limited to frog oocytes, such as *Xenopus laevis* oocytes, and the like. Suitable invertebrate cells include, but are not limited to insect cells, such as *Spodoptera frugiperda* (Sf9) cells, and the like. Any cell known in the art which may be transfected to transiently or constitutively express the transport protein are suitable for use in the present invention.

Page 7, first full paragraph:

A2 A suitable substantially identical protein is a protein having an amino acid sequence that is generally at least 90% identical to the amino acid sequence of human TREK-1 (SEQ ID NO:2). More preferably, the protein is at least 95% identical to SEQ ID NO:2. Most preferably, the amino acid sequence is at least 99% identical to SEQ ID NO:2.

Paragraph bridging Pages 7 and 8:

A3 The coding sequence of the potassium transport protein may be inserted between the noncoding sequences 5' and 3' of a *Xenopus laevis* protein (such as globin) in an appropriate vector, such as pEXO. The construct is introduced into an appropriate cell type to replicate the vector and/or to transcribe RNA. Alternatively, the vector may be used as a template for *in vitro* transcription. A complementary RNA (cRNA) is transcribed and injected into a cell, such as a *Xenopus* oocyte. Such a procedure may be performed in a 0.3 ml perfusion

A3 chamber, wherein single oocytes are impaled on two standard glass microelectrodes (0.5-2.0 MW) charged with 3 M KCl and maintained under voltage clamp with a Dagan TEV200 amplifier. The bath solution contains 98 mM KCl, 1.8 mM CaCl₂, 2 mM MgCl₂, and 5 mM HEPES at pH 7.4 with KOH.

Page 8, first full paragraph:

A4 Alternatively, functional expression of the potassium channel may be accomplished by transfection of insect cells, such as *Spodoptera frugiperda* (Sf9) cells. Briefly a suitable vector, such as pVL1392 may be used and the coding sequence for the potassium transport protein may be inserted into the vector in-frame so that expression of the potassium transport protein may be expressed. The coding sequence for the potassium transport protein may be obtained by any convenient method, such as by PCR or by digesting a plasmid containing the potassium transport protein coding sequence with appropriate restriction endonuclease(s) for subsequent ligation into the pVL1392 vector. Similarly, the amplified product of the PCR may be digested with restriction enzymes and ligated into the vector. Transfection of Sf9 cells may be performed by the manufacturer's protocol (Pharmingen).

Paragraph bridging Pages 8 and 9:

A5 The invention will be described in greater detail with reference to the examples which are provided to illustrate the invention. The examples are not to be construed to be limiting as to the scope of the invention, which is set forth in the appended claims.

Paragraph bridging Pages 9 and 10:

A6 Due to the degeneracy of the DNA code, it will be well understood to one of ordinary skill in the art that substitution of nucleotides may be made without changing the amino acid sequence of the protein. Therefore, the invention includes any nucleic acid sequence for the human TREK-1 channel that encodes the amino acid sequence determined for human TREK-1 (SEQ ID NO:2). Moreover, it is understood in the art that for a given protein's amino acid sequence, substitution of certain amino acids in the sequence can be made

without significant effect on the function of the protein. Such substitutions are known in the art as "conservative substitutions." The invention encompasses human TREK-1 proteins that contain conservative substitutions, wherein the function of the protein is not altered.

AG Generally, the identity of such a mutant TREK-1 will be at least 90% identical to SEQ ID NO:2. Preferably, the mutant TREK-1 will be at least 95% identical to SEQ ID NO:2. More preferably, the mutant TREK-1 will be at least 97% identical to SEQ ID NO:2. Most preferably, the mutant TREK-1 will be at least 99% identical to SEQ ID NO:2.

Page 10, first full paragraph:

A7 The sequence for murine TREK-1, which is a corrected form of murine TREK-1 reported earlier, is shown in SEQ ID NO:4. There is a longer open reading frame than originally reported, producing a protein with a deduced amino acid sequence of 411 amino acids. Due to the degeneracy of the DNA code, it will be well understood to one of ordinary skill in the art that substitution of nucleotides may be made without changing the amino acid sequence of the protein. Therefore, the invention includes any nucleic acid sequence for the murine TREK-1 channel that encodes the amino acid sequence determined for murine TREK-1 (SEQ ID NO:4). Moreover, as is the case with human TREK-1, it is understood in the art that for a given protein's amino acid sequence, substitution of certain amino acids in the sequence can be made without significant effect on the function of the protein. The invention encompasses murine TREK-1 proteins that contain conservative substitutions, wherein the function of the protein is not altered. Generally, the identity of such a mutant TREK-1 will be at least 90% identical to SEQ ID NO:4. Preferably, the mutant TREK-1 will be at least 95% identical to SEQ ID NO:4. More preferably, the mutant TREK-1 will be at least 97% identical to SEQ ID NO:4. Most preferably, the mutant TREK-1 will be at least 99% identical to SEQ ID NO:4.

Paragraph bridging Pages 12 and 13:

A8 The actual concentrations of anesthetics were subsequently determined by means of

A8 a gas chromatography method (HP 6890 equipped with a DB624 column) using FID detection. Samples (2.5 ml of solution) were collected prior to (t_0) and after perfusion (t_{45}) through the experimental setup. Solutions were collected using gas impermeable tubing and stored in sealed glass containers at 4°C for subsequent analysis. Samplings and measurements were performed in duplicate. Actual concentrations of anesthetics were determined by multiplying the calculated concentration by the ratio t_{45}/t_0 (chloroform: 0.16; halothane: 0.37, isoflurane: 0.76; and diethyl ether: 0.57). In the dose effect curves, the threshold concentrations were estimated as concentrations producing an increase higher than 10% in current amplitude.
